

**[0086] B. Fractionation Range**

**[0087]** Objects separated by size on microfluidic devices include cells, biomolecules, inorganic beads, and other objects. Typical sizes fractionated range from 100 nanometers to 50 micrometers. However, larger and smaller particles may also sometimes be fractionated.

**[0088] C. Volumes**

**[0089]** Depending on design, a device or combination of devices might be used to process between about 10  $\mu$ l to at least 500  $\mu$ l of sample, between about 500  $\mu$ l and about 40 mL of sample, between about 500  $\mu$ l and about 20 mL of sample, between about 20 mL of sample and about 200 mL of sample, between about 40 mL of sample and about 200 mL of sample, or at least 200 mL of sample. Total volume of material processed can, in some instances be between 50 ml and 5000 ml. 100 ml and 4000 ml or 500 and 2000 ml. Starting materials include blood, preparations derived from blood (e.g., apheresis or leukapheresis preparations), other biological fluids or extracts, cells grown in culture, etc.

**[0090] D. Channels**

**[0091]** A device can comprise one or multiple channels with one or more inlets and one or more outlets. Inlets may be used for sample or crude (i.e., unpurified) fluid compositions, for buffers or to introduce reagents. Outlets may be used for collecting product or may be used as an outlet for waste. Channels may be about 0.5 to 100 mm in width and about 2-200 mm long but different widths and lengths are also possible. Depth may be 1-1000  $\mu$ m and there may be anywhere from 1 to 100 channels or more present. Volumes may vary over a very wide range from a few  $\mu$ l to many ml and devices may have a plurality of zones (stages, or sections) with different configurations of obstacles.

**[0092] E. Gap Size (Edge-to-Edge Distance Between Posts or Obstacles)**

**[0093]** Gap size in an array of obstacles (edge-to-edge distance between posts or obstacles) can vary from about a few (e.g., 1-500) micrometers or be more than a millimeter. Obstacles may, in some embodiments have a diameter of 1-3000 micrometers and may have a variety of shapes (round, triangular, teardrop shaped, diamond shaped, square, rectangular etc.). A first row of posts can be located close to (e.g. within 5  $\mu$ m) the inlet or be more than 1 mm away.

**[0094] F. Stackable Chips**

**[0095]** A device can include a plurality of stackable chips. A device can comprise about 1-50 chips. In some instances, a device may have a plurality of chips placed in series or in parallel or both.

**EXAMPLES**

**[0096]** The following example is intended to illustrate, but not limit the invention.

**[0097]** A normal blood sample was diluted to 0.2 $\times$  and processed on the DLD in a normal separation mode (running sample against buffer). The initial product fraction collected had the expected concentration based on the input counts and the DLD device ratios of inlets and outlets (further 0.28 $\times$  dilution). Mid-way through the run, the collected product was recirculated into the DLD device as the "wash stream." Various fractions were collected at different time points as the DLD product continued to be recirculated. The final volume and concentration of the DLD product was compared to the input volume and concentration of the sample in order to obtain a recovery value. The net recovery of WBCs after multiple passes was 97.5%, and the concen-

tration factor change from input material to product material was 2.9 $\times$  (versus the initial change of 0.28 $\times$  from input to product when processing the sample against running buffer).

**[0098]** All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by one of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

1. A method of separating target cells or target particles of a predetermined size from a sample comprising cells or particles of less than the predetermined size, the method comprising:

- a) applying both the sample and a wash fluid to a microfluidic device at separate inlets, wherein:
  - i) the wash fluid applied to the device is devoid of said target cells or target particles and devoid of said cells or particles of less than the predetermined size;
  - ii) the microfluidic device comprises an array of obstacles arranged in rows, with each subsequent row of obstacles shifted laterally with respect to a previous row, and wherein the obstacles are positioned so as to differentially deflect target cells or particles to a first outlet where they may be recovered as a target cell or target particle product, and to direct the cells or particles of less than the predetermined size to a second outlet where they may be collected or discarded as waste;
- b) performing deterministic lateral displacement (DLD) by flowing the sample and wash fluid through the device, wherein during the performance of said DLD, at least a portion of the target cell or target particle product is recirculated one or more times so as to replace, all, or at least a portion, of the wash fluid being applied to the device;
- c) during, or at the end of, step b), collecting a final product comprising target cells or particles from the first outlet.

2. The method of claim 1, wherein after the target cell or target particle product has been recirculated, recirculation is stopped and wash fluid is again applied to the microfluidic device.

3. The method of claim 1, wherein the wash fluid is water or an aqueous buffer, and/or a) comprises reagents that chemically react with cells, particles or other components in the wash fluid; or b) comprises antibodies, carriers or activators that interact specifically with target cells or target particles.

4. The method of claim 1, wherein the first outlet comprises, or is connected to, a valve that can be used to divert the target cell or target particle product to a conduit that recycles the product to an inlet on the microfluidic device.

5. The method of claim 1, wherein the microfluidic device comprises an inlet that comprises or is connected to a valve that can be used to switch the feed entering the device through the inlet from a conduit feeding wash buffer to a conduit feeding cell or target particle product.

6. The method of claim 1, wherein the target cell or target particle product being recirculated to said microfluidic device is reacted with, or bound to, a carrier, antibody, fluorescent tag, activator or compound prior to, during or after being reapplied to the microfluidic device.

7. The method of claim 1, wherein cell or particle counts are made of the target cell or target particle product.